population. This is an extreme case of a continuously varying species, the end members overlapping but not inter-breeding.

The museum man with large collections of specimens from all over the world is in a favourable position for studying this type of geographical distribution; but it is the field-worker who can best study local variations of physiological characters, and these, being more intimately concerned with the animals' welfare, are often of great adaptive value. The museum worker is constantly finding differences between his specimens and being unable to decide whether these are due to the effects of the environment or not; only someone in the field can find out for him.

The president of Section D (Dr. C. F. A. Pantin), Mr. J. Colman, Mr. H. C. Gilson, Prof. H. R. Hewer, Prof. A. D. Hobson and Dr. C. F. Hickling took part in the discussion which followed. Dr. Pantin drew a distinction between biology and the physical sciences; workers in either research or teaching in biology have to derive general principles from very complex situations. The solution of biological problems, he said, often comes from unexpected directions, and can only be appreciated by those with a direct contact with the animals themselves.

TRANSFER OF LIGHT ENERGY WITHIN THE PIGMENT SYSTEMS PRESENT IN PHOTOSYNTHESIZING CELLS

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IN investigations[1,2] on photosynthesis of diatoms, it was found that light quanta absorbed by the carotenoid fucoxanthin brought about as strong a fluorescence of chlorophyll *a* as light absorbed by chlorophyll *a* itself; thus, energy transfer occurs with an efficiency of one hundred per cent.

With more refined spectral methods, we have carried out a more general investigation of the energy transfer between pigments within the photosynthetic apparatus. The work covers the main groups of photosynthesizing organisms. Fluorescence spectra were determined by means of a monochromator and a slightly modified photoelectric A.C. amplifier as described by Milatz and Bloembergen[3]. Various wave-lengths of incident monochromatic light were used for exciting fluorescence; the absorption of the incident light in the cells was also determined. Action spectra of photosynthesis of the same organisms were determined by J. C. Goedheer in this Laboratory, using a polarographic method[4]. In some cases phototaxis action spectra, which may be assumed to correspond with the photosynthesis action spectra[5,6], have been measured with Manten's bacteriophotometer[5].

The main photosynthetic pigment in purple bacteria, bacteriochlorophyll, probably occurs in three distinct bacteriochlorophyll-proteins[7], distinguished by absorption maxima at 800, 850 and 890 mμ respectively. For the sake of convenience they will be called here *B* 800, *B* 850 and *B* 890.

By examining fluorescence spectra, it was found that only *B* 890 shows fluorescence.

Transfer of light energy from the carotenoids to *B* 890 was found to occur in *Chromatium* strain *D* and in a strain of *Rhodospirillum molischianum* with efficiencies of 35–40 and 50 per cent respectively. If complete transfer to *B* 890 occurred, the fluorescence action spectrum (corrected for absorption) would be proportional to the absorption spectrum of the bacteria, also between 450 and 550 mμ, in which region the absorption is mainly due to the carotenoids. If, on the other hand, no energy transfer to *B* 890 occurred, the fluorescence action spectrum would be proportional to the absorption spectrum of bacteriochlorophyll, and only a small activity for fluorescence excitation would be found between 450 and 550 mμ. The fluorescence action spectrum actually found (Fig. 1) proves that a partial transfer takes place. Fig. 1 further shows that the action spectrum for *B* 890-fluorescence is proportional to the action-spectrum for phototaxis (or photosynthesis). This indicates that the carotenoids take part in photosynthesis only by transferring their excitation energy to *B* 890.

Additional experiments in the infra-red region showed that *B* 800 and probably *B* 850 also transfer their excitation energy to *B* 890.

By determining fluorescence spectra for various wave-lengths of incident light, it was found that, in vital cultures of *Chlorella*, only chlorophyll *a* shows fluorescence. From the fluorescence action spectrum it could be concluded, in an analogous way as above, that an almost complete transfer of excitation energy from chlorophyll *b* to chlorophyll *a* occurs, which is in agreement with the high efficiency of chlorophyll *b* in photosynthesis.

In solutions of about 10^{-3} *M* of both pigments in acetone, transfer of energy from chlorophyll *b* to *a* was also established, the efficiency being about 50 per cent.

The rather complicated fluorescence spectra of the red algæ *Porphyra lacineata* and *Porphyridium cruentum* and also of the blue alga *Oscillatoria* sp. could be analysed reasonably well in terms of fluorescence spectra of chlorophyll *a*, of the phycobilins and of an unknown pigment with a fluorescence maximum at about 725 mμ.

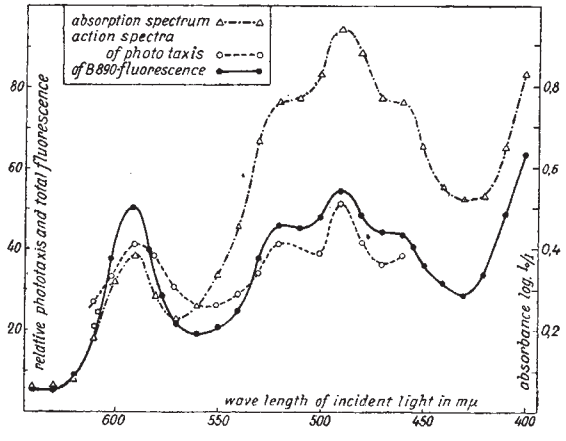

Fig. 1. Absorption spectrum, and action spectra for phototaxis and for *B* 890-fluorescence of *Chromatium* strain *D*. The absorption peak at 590 mμ is due to bacteriochlorophyll, the absorption between 450 and 550 mμ mainly to carotenoids. The carotenoids appear to be active with about the same efficiency both for phototaxis and for bacteriochlorophyll fluorescence. The fluorescence molecule is *B* 890 (see text)

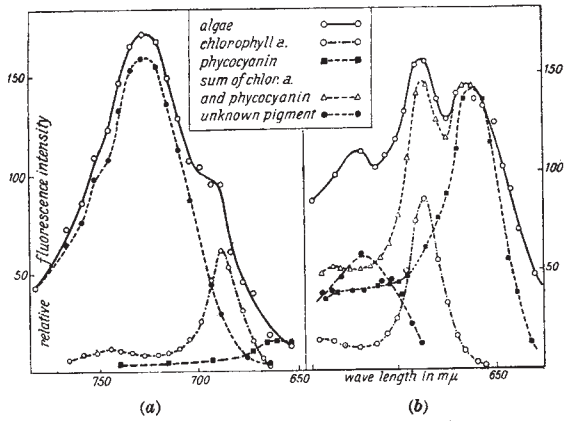

Fig. 2*a*. Fluorescence spectrum of *Porphyra lacineata*; relative fluorescence per quantum of incident light absorbed by chlorophyll *a* at 420 mμ (70 per cent of the absorption is due to chlorophyll *a*)

Fig. 2*b*. Fluorescence spectrum of *Porphyra lacineata*; fluorescence per quantum absorbed by all pigments at 546 mμ (approximately 70 per cent of the total absorption being due to phycoerythrin)

The fluorescence of chlorophyll *a* in *Porphyra lacineata* (Fig. 2*b*) per quantum absorbed by all pigments (incident light of wave-length 546 mμ) appears to be stronger than the chlorophyll *a* fluorescence (Fig. 2*a*) per quantum absorbed by chlorophyll *a* itself (incident light of wave-length 420 mμ). As the absorption at 546 mμ is mainly due to the phycobilins and chlorophyll *a*, it follows that quanta absorbed by the phycobilins excite chlorophyll *a* molecules to a stronger fluorescence than quanta absorbed by chlorophyll *a* itself. This surprisingly high yield of chlorophyll *a* fluorescence for light absorbed by the phycobilins, as compared with the yield for light absorbed by chlorophyll *a*, was also found—but still more pronounced—in the other species of blue and red algæ investigated.

From these observations it may be concluded, not only that chlorophyll *a* occurs in these cells in two different modifications, differing in fluorescence yield, but also that energy is transferred from the phycobilins to the highly fluorescent part of chlorophyll *a*, probably with high efficiency, and that consequently energy is not, or only to a slight degree, transferred to the weakly fluorescent chlorophyll *a* molecules. The weak fluorescence of these molecules in *Porphyra* may be due to transfer of energy to the above-mentioned unknown pigment, since its fluorescence is particularly strong (Fig. 2*a*) for the wave-lengths of incident light for which chlorophyll *a* fluorescence is comparatively low. The following observations support this view. By preliminary spectral and chromatographical investigations of *Porphyra* extracts carried out by F. van de Veerdonk in this Laboratory, a pigment was found—there are indications that it is chlorophyll *d*—of which the absorption was only about one-thousandth of that of chlorophyll *a* at the respective maxima of absorption. If we assume that this pigment occurs in *Porphyra* in the same ratio to chlorophyll *a* as in the extracts, its absorption is too small to be responsible for the strong fluorescence found. Therefore this fluorescence can only be due to energy-transfer from another pigment.

Energy transfer was also established in the red algæ from phycoerythrin to phycocyanin with an efficiency of probably more than 80 per cent in *Porphyridium cruentum*, and also from phycocyanin to chlorophyll *a* with about the same efficiency as from phycoerythrin. In these experiments, the wave-lengths of incident light exciting the fluorescence were: 546 mμ, mainly absorbed by phycoerythrin, and 600 mμ, mainly absorbed by phycocyanin.

Prof. C. S. French, working with *Porphyridium* at about the same time, kindly sent me action spectra, for incident light in the region 400–550 mμ, for the fluorescence of chlorophyll *a*, phycoerythrin, and phycocyanin. The action spectrum for chlorophyll *a* fluorescence demonstrates in an elegant way the low yield which occurs in the region in which chlorophyll *a* is mainly absorbing, as compared with the region in which phycoerythrin is mainly absorbing. The action spectra suggest, as Prof. French remarks, that light energy is transferred from phycoerythrin via phycocyanin to chlorophyll *a*. Hence our results seem to be in good accordance with his.

Light absorbed by the phycobilins proved to be more active in photosynthesis than light absorbed by chlorophyll *a*, as was previously established by Haxo and Blinks[4] for other species of red and blue algæ. Owing to experimental difficulties, hitherto we could only establish qualitatively the parallelism between the yield of chlorophyll *a* fluorescence and photosynthesis in red and blue algæ. Considering the position in other groups of organisms already described, we put forward the following picture of the two paths of energy transfer in red algæ:

(1) Phycoerythrin → phycocyanin → chlorophyll *a* → photosynthesis
↘ fluorescence ↘ fluorescence ↘ fluorescence and other energy losses.

(2) Weakly fluorescent chlorophyll *a* → unknown pigment → fluorescence and other energy losses.
↘ heat and other energy losses

In the blue alga *Oscillatoria* the path of energy transfer was found to be the same, the only difference being that phycoerythrin is lacking. It should be emphasized, however, that in *Porphyra* the fluorescence of the unknown pigment is more pronounced than in the other species of red and blue algæ; so the energy transfer from the weakly fluorescent part of the chlorophyll *a* to this pigment is only assumed on grounds of analogy.

The transfer of electronic excitation energy between pigment molecules as reported above probably takes place through inductive resonance between the excited molecules and the molecules in the ground-state, a theory for which Förster[8] has given a quantum-mechanical treatment, with the aid of which the probability of energy transfer can be calculated from experimental data. Estimations, based on Förster's considerations, are in accordance with, or at least do not contradict, the results recorded above.

Summarizing, we may say that the evidence obtained is in favour of the view that, in algæ, light energy absorbed by a pigment, active in photosynthesis, is transferred to the 'chemical' part of photosynthesis only via chlorophyll *a*, and in purple bacteria only via bacteriochlorophyll *B* 890.

A detailed report of this and related work will be published elsewhere.

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[1] Dutton, H. J., Manning, W. H., and Luggar, B. H., *J. Phys. Chem.*, **47**, 308 (1943).
[2] Wassink, E. C., and Kersten, J. A. H., *Enzymol.*, **12**, 3 (1946).
[3] Milatz, J. M. W., and Bloembergen, N., *Physica*, **9**, 449 (1946).
[4] Haxo, F., and Blinks, L. R., *J. Gen. Phys.*, **33**, 389 (1950).
[5] Manten, A., thesis, Utrecht (1948).
[6] Thomas, J. B., *Biochem. et Biophys. Acta*, **5**, 186 (1950).
[7] Wassink, E. C., Katz, E., and Dorrestein, R., *Enzymol.*, **7**, 113 (1939).
[8] Förster, Th., *Ann. Physik*, **2**, 55 (1948).

CONFIGURATION OF POLYPEPTIDE CHAINS

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We have recently formulated a number of configurations of polypeptide chains that differ from all those previously discussed in that they conform rigorously to the structural principles that have been set up on the basis of the precise X-ray studies of crystals of amino-acids, simple peptides, and related substances that have been made in our Laboratories during the past fifteen years[1-3,5,8]. In the proposed configurations, the interatomic distances and bond angles have the values found experimentally, the amide groups are planar, and hydrogen bonds N—H...O are formed with the N—O distance 2·8 A. and with the oxygen atom close to the N—H axis. Agreement in dimensions of units of structure and in observed and calculated intensities of X-ray diffraction maxima has provided strong evidence that these configurations are present in various synthetic polypeptides[4] and fibrous proteins[6-8], and also in hæmoglobin[9].

Perutz has recently suggested[10] a way of testing for the presence of our 3·7-residue helix, which he describes as "a simple and decisive criterion in the X-ray diffraction pattern". In the 3·7-residue helix (which we call the α-helix) there are approximately 3·7 residues per turn of the helix, which has a pitch of about 5·55 A., and thus the axial length per residue is 1·5 A. Perutz has pointed out that the form factor for the helix would show a strong maximum in the meridional direction at the spacing 1·5 A., and that accordingly synthetic polypeptides and proteins with this helical structure would be expected to produce a diffraction maximum at the spacing 1·5 A., if the specimen be suitably oriented. He found that the reflexion is given by poly-γ-benzyl-L-glutamate, hair, and porcupine quill-tip, but not by hair stretched to the β-form. He concluded that the "presence of this reflexion does not in itself prove the correctness of Pauling and Corey's structure, but taken in conjunction with the favourable agreement of observed and calculated intensities of other reflexions already obtained by these authors, it leaves little doubt about their structure being right". It should be mentioned that we had previously mentioned the existence and significance of the 1·5-A. reflexion, in the following words (ref. 7): "We would expect that side chains of different kinds on the α helix would repeat after an integral number of residues, corresponding to an integral multiple of the residue length along the helix axis, about 1·53 A, and that accordingly those orders of basal plane reflection for which the spacing approximated closely to certain multiples of 1·53 A. would be enhanced in intensity. It is in fact found that about 80 per cent of the meridional reflections are of this type; for both Venus clam muscle and porcupine quill they are multiples of 1·51 A. The reflections at 1·49 A, 3·05 A, 4·50 A, and 6·19 A for porcupine quill, which represent the first four orders of enhancement, are the strongest features of the wide-angle meridional pattern, except for the 5·2-A arc."

Perutz also looked for the 1·5-A. reflexion on X-ray photographs of feather rachis keratin, for which we suggested a structure involving 3·7-residue helixes as well as pleated sheets of extended chains[6], and he states: "Prolonged exposure of seagull's feather rachis set at the appropriate Bragg angle revealed no trace of such a reflexion, which shows that the structure proposed by Pauling and Corey must be wrong". (We may add that we have recently discovered a type of pleated sheet differing somewhat from that described in our earlier papers; this new pleated sheet, with alternate chains reversed in direction, might be present in feather rachis keratin and other proteins, instead of the polar pleated sheet.)

We agree with Perutz that the 1·5-A. reflexion is an important criterion for the 3·7-residue helix, but our assessment of the significance of the absence of this reflexion differs from that of Perutz. We agree with him that the presence of the reflexion does not in itself rigorously prove the presence of the 3·7-residue helix, but that a strong reflexion in the right position relative to the fibre axis direction and with spacing in the range 1·46–1·55 A. is to be taken as very strong evidence of the presence of the α-helix. On the other hand, Perutz, as shown by his statement about feather rachis quoted above, has assumed that the absence of such a reflexion rigorously eliminates the α-helix from consideration. This is not true. For example, a protein or synthetic polypeptide might have a structure involving α-helixes packed together in such a way that half the helixes are displaced along the fibre axis relative to the other half by an odd multiple of 0·75 A. In this case the radiation scattered by one chain in the direction giving rise to the 1·5-A. reflexion would be exactly out of phase with the radiation scattered by a neighbouring chain, and interference would result which would cause the intensity of this reflexion to be zero. We hence conclude that the presence of the 1·5-A. reflexion is to be taken as strong evidence of the presence of polypeptide chains with the 3·7-residue helical configuration, but that the absence of this reflexion is not to be taken as strong evidence of the absence of the 3·7-residue configuration; it indicates either that these helixes are not present, or that they are present but are arranged in such a way as to produce strong inter-chain interference for this reflexion.

Let us consider as an example the crystalline synthetic polypeptide poly-γ-benzyl-L-glutamate, which we have described[4], on the basis of data published by Bamford, Hanby and Happey[11], as having a monoclinic (pseudo-orthorhombic) structure involving two polypeptide chains per unit, these chains having the 3·7-residue configuration, with eleven residues in three turns. The two chains in the unit